

Conference Presentation

## High sensitive materials in medical holographic microscopy

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## High sensitive materials in medical holographic microscopy

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**Abstract.** High sensitivity is defined in relation to the energy required to perform holographic recording. High sensitivity in silver halide materials is their main advantage over other similarly high resolving power holographic recording materials. This work reports progress on the development of silver halide based ‘true colour holographic imaging’, under a microscope. A thin layer of ultrafine grains of silver halide crystals of around 10 nm average diameter, dispersed in a colloid and coated on a substrate is used as the recording media. The significance of this method so far, is in its ability to produce ‘true colour’ three-dimensional images of specimen. The recordings have an appreciable depth, permitting the observer to scan through the image under a microscope, as one might with a real specimen sample. Current methods could perform ‘True colour holographic imaging’ directly under a microscope. The recording methodology has the potential for deeper complex and scattering media imaging, using very small pulses of appropriate laser wavelengths. The methodology, using novel nano-size panchromatic recording media consisting of dispersed fine nano grain crystals, could potentially revolutionise related medical imaging techniques. Future development of digital media will allow it to be utilized in this manner.

### 1. Introduction

Holographic microscopy in cellular imaging has emerged in recent years as a new tool for medical imaging application [1-16]. Current techniques allow imaging of very thin layers of scattering media. Stepping up these techniques to apply them for medical research is technologically challenging. The current generation of digital image sensors and spatial light modulators are limited in dealing with light diffraction [17].

This work reports on the development of ‘True colour optical biopsy’. Rather than using digital imaging devices, a thin layer of ultrafine grain of silver halide crystals of 10-20nm average diameter, dispersed in a colloid and coated on a substrate is used as the recording media. The significance of this method so far, is in its ability to produce three-dimensional true colour images of specimen. They have an appreciable depth, permitting the observer to scan through the microscopic image, as one might with a real sample of cells, e.g. from biopsy. Current methods could ‘True colour optical biopsy’ the skin, the gastrointestinal tract and anywhere accessible with a direct scope imaging. This has the potential for deeper tissue holographic ‘True colour optical biopsy’ using very small pulses of appropriate laser wavelengths.



The hologram is a pattern of fringes formed by the interference of light scattered from the object and light which comes from the point reference source. The pattern contains total three-dimensional information of a scene, gathered in a single exposure. This provides a unique opportunity in microscopy in comparison to alternative microscopy methods, such as confocal microscopy or the traditional compound microscopy. Achieving this, requires considerations be given to; (i) the resolving ability of the recording media, to record the highest spatial frequencies of the interference pattern created, and (ii) that the theoretical resolution is limited by diffraction. Recording a scene requires two light beams, of wavelength  $\lambda$ , to meet at the recording media at an angle  $\theta$ . They form fringes of pitch  $\lambda/(2n \sin(\theta/2))$ . A small  $\theta$  may result in noise in the form of zero order becoming difficult to separate from the true image. Phase shifting methods attempt to overcome this problem at a minimum expense [18, 22]. But, as  $\theta$  approaches  $180^\circ$ , higher resolution recording media is necessary to resolve the fine fringes produced. For a pixel of size  $X$ , the maximum wave number  $\zeta_{\max}$  that can be resolved is the inverse of twice the pixel size, *i.e.*  $\zeta_{\max} \sim 1/(2X)$  and  $\zeta = \sin(\theta)/\lambda$ . This limits the maximum angle between the reference and object beams. For example, for a device pixel size of  $1.4\mu\text{m}$ , and a red beam of wavelength  $633\text{nm}$ , the maximum angle would be  $\theta_{\max} = \sin^{-1}((633 \times 10^{-9}) / (2 \times 1.4 \times 10^{-6})) \sim 13^\circ$ . This limit can oppose the common diffraction resolution limit of the order of  $z\lambda/a$ , where  $z$  is the imaging distance and  $a$  is the aperture size. Reducing the distance  $z$ , between the sensor and the object, to improve resolution, results in fringe patterns too fine to be resolved by the imaging pixels. Much of the effort in digital holographic imaging is to compensate for related insufficiencies [17]. Most active technological efforts also include colour imaging, important in microscopy [21]. Holographic 'True colour optical biopsy' using suitable lasers and novel nano-size panchromatic recording media consisting of dispersed fine nano grain crystals, could potentially revolutionize surgical techniques. Future development of digital recording media will allow it to be utilized in this manner.

## 2. A flexible light laser engine and delivery system

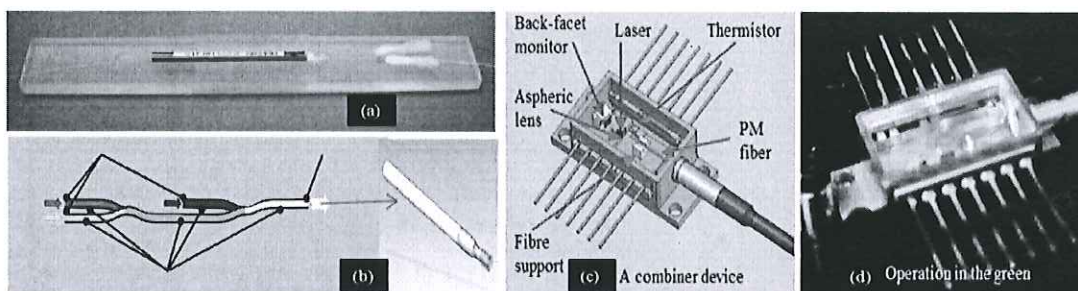
### 2.1. Industrial tasks

Flexible, mobile, true colour holographic endoscopy requires a fibre delivered system with both suitable visible laser wavelengths combination and PM (Polarization Maintaining) transmission, preferably integrated in a single device. Gooch and Housego fibre optics laboratories (G&H) [30], with expertise to manufacture both RGB (red, green, and blue) sources and fused PM combiners were tasked with the development of a combination device with: (i) stable coherent light sources, operating at around 20mW in the red, green and blue regions at 658nm, 532nm and 452nm respectively; (ii) an output PER (Polarization Extinction Ratio) of greater than 15dB; and (iii) a transmission between 70% and 90% of each visible region. The final delivery system outputting RGB laser light through a PM Cable requires PM combining devices with SM inputs and a PM output. Figure 1(a) shows an example device fixed to an external substrate, and under green laser light operation. The choice of laser light and other variables were a compromise based on availability and suitability. Fabricating a low loss device that is both single-mode and polarization maintaining for all three RGB wavelengths proved challenging, but critical for a flexible holographic endoscopic imaging device. Figure 1(b), illustrates a simplified schematic of the device attached to a prospective endoscopic unit.

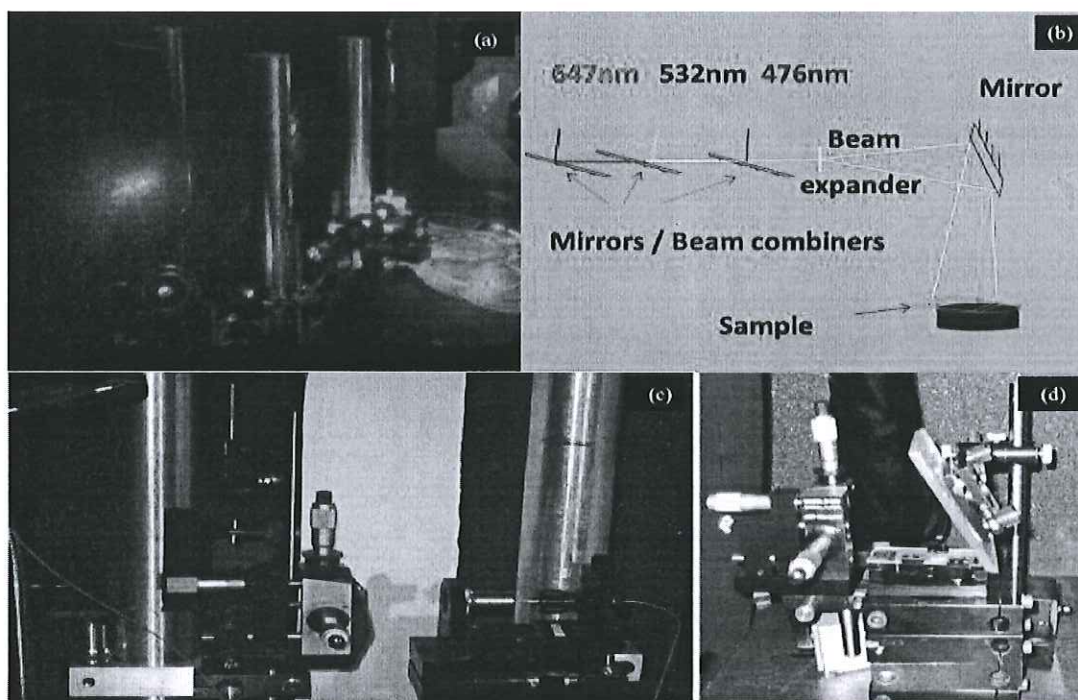
### 2.2. A subsection

As a direct result of this study, early innovations in fused fiber developments resulted in a G&H patent application US7653269B1. The methodology involves the use of a short stub of (non-PM) SM fiber which transmits blue light with low loss, as the "mixer region" in a fused coupler with PM inputs and outputs. The resulting world-first device with the combined output having a polarization extinction of 7dB was an excellent innovative achievement, however holographic 'True color optical biopsy' demands achieving greater than 15dB of polarization extinction at all three RGB wavelengths. In regard to progress with the required RGB PM lasers, so far three types of pigtailed single mode PM

laser have been developed: all commercial diodes pigtailed using in-house techniques and materials in an industry standard “14-pin butterfly” package, Figure 1(c), with integrated power supply as a “stand-alone” device. Figure 1(d) demonstrates a green, 532nm, PM pigtailed laser, using a frequency converted 808nm laser diode as the fundamental source, in a butterfly package. It has the ability to achieve stable fiber-coupling of up to 20mW and a polarization extinction of about 20dB. The blue 452nm pigtailed laser with greater than 20dB produced 5mW, but the red (660nm) pigtailed laser with a PER of greater than 20dB produced more than 40mW. The developments have been of industrial relevance in terms of original work relating to commercial success, particularly in the case of the 660nm pigtailed laser, leading the industrial partners to develop closely related devices for demands in the UK and globally.



**Figure 1.** Images supplied courtesy of Gooch and Housego (G&H), (a) A PM combiner device with two SM inputs and a PM output, fixed to an external substrate, under green light operation, (b) a simplified schematic of the device attached to an endoscopic unit that should encapsulates a film holding unit, and (c, d) An industry standard “14-pin butterfly” package, operating in the green.

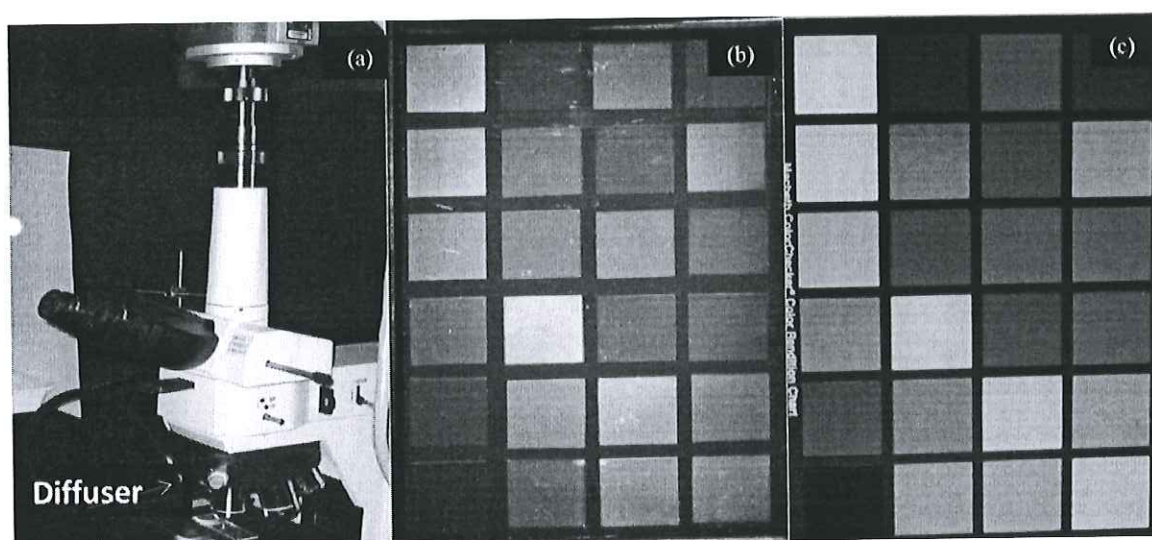


**Figure 2.** (a) The in-vitro recording setup, (b) schematic, (c) flexible beam delivery system for (d) delivery to the recording sample through a flat front silver mirror.

### 3. True colour recording

#### 3.1. Novel recording media

Over the last few years, suitable low scatter panchromatic emulsions having a diffraction efficiency of around 95%, have been emerging. For example, the SilverCross material developed in house at Glyndŵr University [23] and used for true colour recordings reported in [24]. For commercially available recording emulsions, see for example [25-28]. For the recordings in this project, a newly developed version of the standard SilverCross was optimized in terms of large scale produce-ability and sensitivity at a recording exposure of  $\sim 6\text{mJcm}^{-2}$  when machine coated. This compares with a recording exposure of  $\sim 4\text{mJcm}^{-2}$  for the same material when coated by hand, and  $\sim 1.7\text{mJcm}^{-2}$  for a representative commercial contender e.g. the Sphere-S material [25].

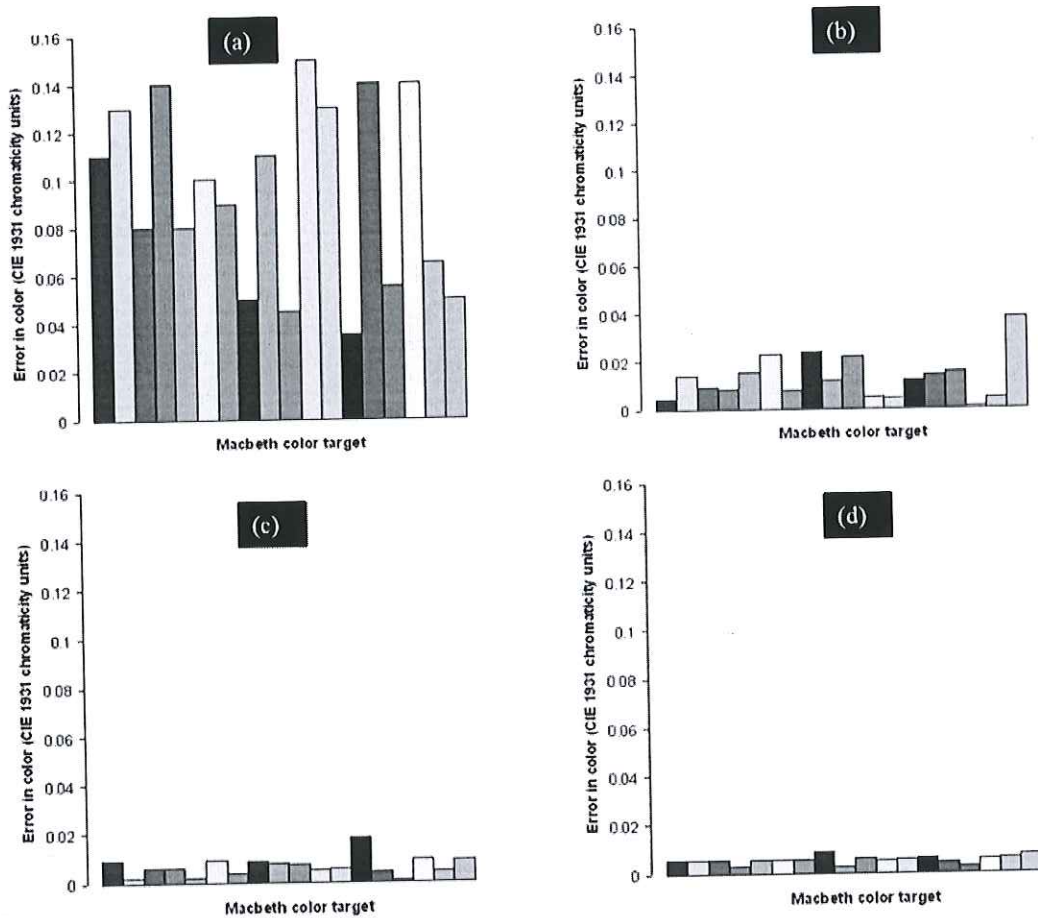


**Figure 3.** (a) “Non-coherent holographic reconstruction” under a microscope, of a (b) recorded hologram of the (c) Macbeth ColourChecker®.

#### 3.2. In-vitro recording setup

The in-vitro setup is a simplified, but panchromatic version of an earlier monochrome in-vivo device, developed at Lake Forest College, dating back several years [31]. Utilizing dye spraying (or vital staining), the device demonstrated high contrast, in-vivo monochrome cellular imaging of the colon of an anesthetized dog. The images, however, lacked colour, particularly important to further aid physicians to distinguish unhealthy and dead tissue. This was the main clinical feedback of the trials. For the current recordings, while waiting for the development of a suitable specifically designed RGB fibre output device, the standard procedure involved panchromatic ultrahigh resolution in-vitro recording. The quality of the recorded colour holograms should be the same, independently of whether they are recorded in an open or fibre-optic holographic setup. The strategy, in the short run, would assist the development of the final holographic system, in demonstrating the best true colour three-dimensional reconstructed images, in terms of:

1. Ability to perform ‘optical biopsy’ of relatively large tissue areas in a single exposure
2. Not requiring extensive training to utilize the recording technique
3. Minimizing experimentation time by the clinical operator
4. Providing repeat examination opportunity e.g. by a specialist pathologist, viewing true colour, three-dimensional, large focal depth, sample image reconstructions.



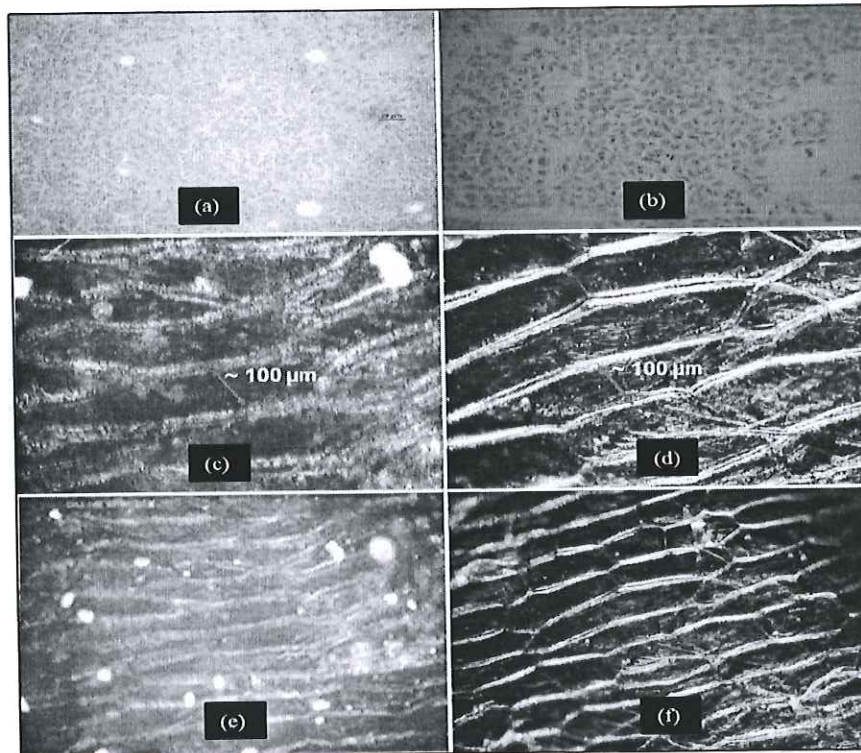
**Figure 4.** Colour error for each Macbeth colour for; (a) three wavelengths, (b) three optimum wavelengths, (c) four wavelengths and (d) five wavelengths.

To achieve these:

1. The setup, Figure 2(a-d), is of a 'white' laser Denisyuk [32] reflection type. The Denisyuk geometry provides maximum viewing angle and full parallax in both directions in the reconstructed images.
2. Keeping the recording media in close proximity to the tissue sample would also allow for minimum operator training and maximum recording area to be exposed to the 'white' wavelengths.
3. Because of the small object distance, the resolution obtained in the hologram is not compromised.
4. The setup contains no focusing element, providing three-dimensional reconstructions that are always 'in focus'.
5. The setup is hence designed to avoid focusing issues associated with small shakes and high magnification.
6. For the proposed recording setup, a commercially available single mode fibre was coupled to the following CW lasers to create the required 'white' beam: krypton ion laser (647 nm), frequency-doubled Nd:YAG (532 nm) and argon ion (476 nm).
7. The choice of visible wavelengths, and all other variables, including the fibre were a compromise based on closeness to optimum recording suitability, availability, and processing convenience [33].

#### 4. Enhanced colour reproduction

Enhanced colour rendering or reproduction is possible if more than the minimum three primary RGB laser wavelengths are used [29]. In-house computer simulation, based on the tri-stimulus theory implications for the broad band light, and the narrow laser wavelengths, using the Macbeth ColourChecker<sup>®</sup> target and assuming a perfect recording media confirms this. The error calculations were based on the total average colour error in CIE 1931 chromaticity units for the optimal number of lasers, for different numbers of lasers, for all possible combinations between 700 and 400 nm [29].



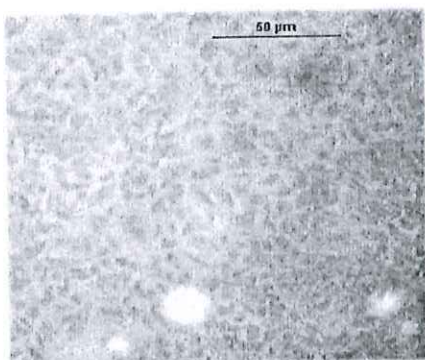
**Figure 5.** (a) ‘True colour optical biopsy’ holographic reconstruction of (b) cultured human endothelial cells. (c) Hologram of (d) onion cells, both at twenty times magnification, also illustrated in (e) and (f) illustrated at ten times magnification.

A non-coherent broadband illumination source ‘MEIJI Techno fibre optic (150W)’ with a ground diffuser added to the beam line Figure 3(a) to enhance speckle reduction effect, was used for all holographic image reconstructions and image assessments. Figure 3(b) shows a non-coherent holographic image reconstruction of the Macbeth ColourChecker<sup>®</sup> Figure 3(c), utilized to check colour reproduction of holographic recordings of the in-vitro recording setup with 647, 532, 476nm wavelengths. The wavelengths were chosen because of availability and closeness to the optimum values defined by the simulation. Figure 4 (a) shows the simulation bar graph displaying the error for each Macbeth colour of the hologram: Figure 4 (b) shows the reduced error, if the optimal RGB wavelengths defined by the simulation 610, 545, 466nm were used. The reduced colour error for the optimum values of four laser wavelengths 620, 571, 518, 459nm is shown in Figure 4 (c), and for five lasers 452, 504, 549, 595, and 643 nm is shown in Figure 4 (d).

#### 5. Colour in-vitro recording

For the holographic recording, Figure 5(a), of the human endothelial tissue, Figure 5(b), samples were cultured, stained, and supplied by expert medical clinicians, in a similar manner to that provided for

medical investigations, *e.g.* by pathologists. Samples were then recorded holographically. Prior to recording endothelial cell samples, test holograms including that of the relatively large size onion cells, Figure 5(c-f), were recorded to assess and calibrate the setup. The medically approved reconstructed colour holographic images, demonstrating resolving cell nuclei, at different surface depth, Figure 6, were successfully investigated by medical experts, on the basis of “added quality information of medical relevance”. Their main criteria were the ability to resolve the cell boundary and the nuclei inside the cells, and doing this at different depths of the surface of the three-dimensional space of the prepared human endothelial sample.



**Figure 6.** Development of medically approved color holograms from the human tissue demonstrating resolving cell nuclei, at different surface depth.

## 6. Discussion

Current methods could enable ‘True colour optical biopsy’ of the skin, the gastrointestinal tract and anywhere accessible with a direct scope imaging. This has the potential for deeper tissue ‘True colour optical biopsy’ using very small pulses of appropriate laser wavelengths. ‘True colour optical biopsy’ using novel panchromatic recording media consisting of dispersed fine nano grain crystals, could potentially revolutionize surgical techniques. Future development of digital recording media will allow it to be utilized in this manner.

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